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Research Article

Claw loss and the prey preferences of an invasive crayfish

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Abstract

Predator-prey relationships underpin the stability of ecosystems but can be perturbed by numerous factors, such as biological invasions. Crayfish readily colonize new ecosystems and their impacts can modify food webs or ecosystem functioning. However, while crayfish exert high levels of predation pressure handling prey with their claws, claw loss is also common in their populations, which could mediate prey selection pressures. Here, we investigate how the number of claws in the marbled crayfish *Procambarus virginalis* modulates its preferences for two different available prey: *Chironomus plumosus* and *Gammarus fossarum*. We showed that the food preference of *P. virginalis* was mainly for soft, less mobile prey, such as *C. plumosus*, with significantly fewer *G. fossarum* (harder, more mobile prey) being killed. This preference was largely independent of claw presence but depended on the availability of prey, with fewer *G. fossarum* being targeted as the number of claws decreased. Our results highlight the complexity of predator-prey ecological relationships in invaded freshwater ecosystems and show that some biotic factors, such as the quantity and type of prey available, need to be taken into account in order to better understand their role in ecosystem dynamics.

Key words: *Chironomus plumosus*, choice experiment, feeding preference, *Gammarus fossarum*, macroinvertebrate community, prey preference, *Procambarus virginalis*

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Introduction

Predator-prey relationships are integral components of trophic interactions and fundamental to the organization and stability of ecosystems, thereby shaping food web structures and regulating energy flows (Levine 1980; Moosmann et al. 2021). The introduction of non-native species can disrupt these relationships through trophic mechanisms, such as predation and competition (Balzani et al. 2016; Doherty et al. 2016; Gaiotto et al. 2020). In recent decades, the unprecedented increase of non-native species' introductions has resulted in drastic food web alteration, resulting in the deterioration of ecosystem functions (Seebens et al. 2017; Mormul et al. 2022). While successful non-native consumers are often omnivorous, their effects are highly context-dependent, as the strength of trophic interactions involving invaders is influenced by factors such as dietary preferences in generalist species (Gallardo et al. 2016).

Crayfish species have been introduced outside of their natural ranges for various purposes (Lodge et al. 2012), including to enhance fisheries and aquaculture (Chucholl 2013; Vodovsky et al. 2017; Haubrock et al. 2021), as well as accidentally through their use as fish bait (Akmal et al. 2023) and deliberately as pets (Patoka et al. 2016; Bláha et al. 2022). When they are introduced into the wild and successfully establish a population, non-native crayfish can constitute the majority of benthic biomass (Momot et al. 1978; Momot 1995) and often cause negative impacts on the recipient ecosystem (Souty-Grosset et al. 2016; Chucholl and Chucholl 2021) through their opportunistic, omnivorous feeding habits (Edmonds et al. 2011; Linzmaier et al. 2020; Veselý et al. 2021). Nevertheless, in recent decades, the implementation of national and international laws aimed at mitigating their negative impacts has led to early signs of saturation in temporal trends, as indicated by a deceleration in growth rates or spread approaching a plateau across Europe (Soto et al. 2023b).

Crayfish chelipeds (hereafter simply 'claws') are involved in many intra- and inter-specific interactions (Fořt et al. 2019; Graham and Angilletta 2020; Roje et al. 2021), sex-specific differences in fighting courtship formation, and mating (Land and Layne 1995; Barki and Karplus 1999; Holdich 2002), burrowing (Kouba et al. 2016; Haubrock et al. 2019), as well as chemoreception (Moore and Belanger 2009; Bauer and Hatt 1980; Kaur et al. 2023). Claws also play a pivotal role in gripping larger, mobile, or hard-shelled prey (Keller and Hazlett 1996; Claussen et al. 2008; Sanders and Mills 2022). Crayfish, similarly to other crustaceans, can voluntarily autotomize their claws while escaping (McVean 1975). They can also lose their claws during aggressive interactions, e.g. territorial fighting between individuals, direct competition for resources, or predatory and non-predatory entrapments such as failed moulting or mating (Bliss 1982; Darnell et al. 2018; Graham et al. 2023).

In general, 10–30% of individuals can exhibit claw autotomy in aquaculture systems (González et al. 2010; Kouba et al. 2011). Powell et al. (1998) observed that under conditions of food decline, up to 33% of the red swamp crayfish *Procambarus clarkii* individuals reared in ponds have at least one claw missing. These values are usually lower in wild populations, yet may remain significant. Up to 34% of individuals exhibited claw autotomy in French white-clawed crayfish *Austropotamobius pallipes* populations (Laurent 1985), and more than 20% were reported for a Turkish population of the narrow-clawed crayfish *Pontastacus leptodactylus* (Berber et al. 2024). At least three-quarters of the population exhibit missing or regenerating claws in the virile crayfish *Faxonius virilis* (Graham et al. 2021; Zackary Graham pers. comm.).

Claw loss results in reductions of an individual's performance and fitness due to impaired feeding, defense, and mating (Mariappan et al. 2000; Kouba et al. 2011; Duermit et al. 2015). Indeed, the number of functioning crayfish claws and the availability of resources (in terms of prey) can affect an individual's consumptive performance (Toutain et al. 2024). However, the ecomorphological and behavioral characteristics of the prey also mediate the strength of the predation (Dobler and Geist 2022; Mpanza et al. 2024), whereby the influence of claws on successful consumption can be profound (cf. Graham et al. 2021). The impact of crayfish predation on ecological communities, therefore, depends on their behavioral flexibility, the food availability and preferences and the number of functioning claws (Spilmont and Seuront 2023, Toutain et al. 2024). If crayfish disproportionately regulate populations of abundant prey, the risk of predation on rare and low-density prey will concomitantly decrease, so prey switching (i.e. a form of frequency-dependent predation)

can facilitate coexistence between prey species in diverse communities. This change in predator-prey interactions may confer stability by limiting predation pressure on low-density prey (Hughes and Croy 1993; Cuthbert et al. 2019). In essence, if a consumer displays prey swichtching, rare resources are disproportionately less consumed, while concurrently, more abundant resources can be more consumed relative to environmental availability (Murdoch 1969; Cuthbert et al. 2019).

It has been demonstrated that, for relatively slow soft-bodied prey, specifically the larvae of the buzzer midge *Chironomus plumosus*, the number of claws in the marbled crayfish *Procambarus virginalis* had a minor effect on their consumption (Soto et al. 2023a). In contrast, the number of claws played a substantial role in the predation of the amphipod *Gammarus fossarum* (Koch, 1836), a more mobile and hard-bodied prey (Toutain et al. 2024). However, beyond these previous pairwise predator-prey experiments, no studies have examined whether the presence or absence of claws mediates prey selectivity in more complex resource communities. Prey preferences can shift rapidly (Alexander et al. 2022), and the absence of claws may acutely prompt a transition towards prey with particular traits by crayfish, such as those with soft over hard bodies. In the present study, we thus compared the predation of non-native crayfish *P. virginalis* on the two above-mentioned prey species, both native and widespread in European freshwaters, keeping the same prey ratio at different total prey densities (6, 12, and 24 individuals). Then, we compared the prey preference at five different prey ratios under the same density (24 individuals) using three groups of crayfish (crayfish with both claws, one claw, and clawless). We hypothesized that the loss of claws (i) would decrease the preference for the hard-bodied prey (i.e. *G. fossarum*), and that (ii) the number of different prey species killed would depend on their availability as well as the number of functioning claws in crayfish.

Methods

Animal collection and maintenance

Experiments were carried out at the Research Institute of Fish Culture and Hydrobiology in Vodňany, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Czech Republic. The marbled crayfish *P. virginalis* (Decapoda, Cambaridae) was chosen due to its severe ecological impacts in freshwater ecosystems, and the availability of similar-sized cohorts in our laboratory facility. The stock of this parthenogenetic species (all females) was kept in a closed recirculating aquaculture system and rearing aquaria fitted with sufficient shelters (e.g. perforated bricks) to minimize stress from intraspecific interactions. Animals were fed daily *ad libitum* using defrosted chironomid larvae, zooplankton, grated carrots, and commercial fish pellets containing microalgae (Sera Granugreen, Sera GmbH, German; Lunda et al. 2020). The light regime was set at 14:10 light:dark, and the water temperature was kept between 21 and 22 °C. We selected a similar-sized group (cephalothorax length ranging from 16 to 21 mm; mean: 18.68 mm ± 1.17 mm) of intermoult non-reproductive crayfish, i.e., without pleopodal eggs or developed glair glands (see Das et al. 2024). Claw autotomy was induced in individuals (~90% of animals with one or without a claw) a few days before the experiment following Soto et al. (2023a). The propodus of the designated claw, specifically in the palm, was pinched by laboratory personnel while the animal remained submerged. This method triggers the caridoid escape

response, simulating natural autotomy conditions. No special authorization is required for inducing autotomy of crayfish claws, beyond institutional standards for animal protection and local jurisdiction. When regenerating claws were present, we performed autotomy on them rather than on fully developed claws, reducing the burden on the animals and maintaining the fully developed claw present in the one-clawed group (see below). Also, autotomy was performed randomly on either the right or left claw when fully developed animals were included in the one-clawed group (see below). The weight of the animals ranged from 1000 to 2600 mg (mean: 1778 mg ± 388 mg). All the crayfish were then sorted into three groups of around 80 individuals, each according to the number of claws and placed in three separate aerated ~ 130 l aquaria (75 × 50 × 35 cm), with a dozen shelters provided (bricks with holes stacked vertically), one for each of the following groups: i) individuals with both claws, with no claw autotomy induced (hereafter 'both claws'), ii) individuals with one claw (left- or right-claw missing; 'one claw') and iii) individuals with no claws ('clawless'). Before their experimental use, animals were acclimatized for at least three days in their respective aquaria.

Similar-sized amphipod *G. fossarum* prey were captured with a fine mesh hand-held net positioned downstream of disturbed stones, logs, and detritus at the Křesanovský brook, Vimperk, Czech Republic (49.0604N, 13.7583E) every three to five days, depending on the experimental needs. Animals were transported to the laboratory and stocked in a 90 l plastic bowl with 40 l of aerated aged tap water at ~ 20 °C. Cylinders made from 1 mm plastic sieves were provided as structures, thereby increasing the habitat complexity and allowing for amphipods' attachment and sheltering. The prey stock was fed with fish pellets containing microalgae (Sera Granugreen); uneaten food, feces, and dead individuals were siphoned, and one-fifth of the water volume was exchanged daily. The individual wet weight and body length (mean ± SD) of a randomly selected subset of *G. fossarum* (n = 30) were 24.17 ± 5.88 mg and 11.72 ± 1.23 mm, respectively. Similar-sized larvae of *C. plumosus* were collected from the mud every two to five days in the experimental ponds of the Research Institute of Fish Culture and Hydrobiology in Vodňany (49.1558 N, 14.1658 E). Prey were stored in 15 l buckets with organic material from ponds. Uneaten food, feces, and dead individuals were siphoned, and one-fifth of the water volume was exchanged daily. The individual wet weight and body length (mean ± SD) of a randomly selected subset of prey (n = 30) were 36.06 ± 6.57 mg and 22.91 ± 3.03 mm, respectively.

Before running trials, each crayfish was independently acclimated for 24 h to the experimental arenas, i.e. an empty plastic box with opaque walls (inner dimensions 26 × 18 × 7.5 cm) filled with 1 l of aged tap water, without food to make them more responsive in the experiments and to standardize hunger among individuals. The arenas were maintained in experimental incubators (Lovibond TC 445 S/445 L, Liebherr, Germany) with a fixed light regime (14:10 light:dark) and a temperature of 20.4 ± 0.59 °C. We chose this temperature because it is a common laboratory regime within the optimum range for the tested crayfish species (Hossain et al. 2019).

Experiment 1: prey choice at equal densities

To assess the effect of claws on foraging activity and prey preference, individual crayfish belonging to the three claw groups were exposed to three different total prey densities (12, 24 and 48), with each of two prey species constituting an equal proportion (i.e. 6:6, 12:12, and 24:24). Both prey types were added simultaneously to the crayfish arena. Each combination was replicated ten times, alongside

four replicates of predator-free controls at each prey density to quantify possible background mortality of the prey. Thus, our experiment consisted of nine treatment combinations (three densities x three claw groups) with a total of 90 independent observations, and 12 controls. The crayfish were allowed to feed for 4 hours, during the light period of the day. The marbled crayfish originate from our laboratory, and our previous assessments of activity and foraging showed notable behavioral peaks during the morning and early afternoon hours - probably corresponding to the periods when routine stock maintenance takes place in the rearing facility. After this period, the number of gammarids and chironomids killed (either consumed or only dead) was assessed by counting the remaining living prey. Prey partially consumed or those showing no signs of life were considered dead, as these conditions represented an impact on these populations and potentially altered trophic dynamics. Crayfish were used only once to prevent habituation effects within the experiment. Live uneaten prey were discarded to prevent potential biases.

Experiment 2: prey preference at different densities

To assess the effect of claws on foraging activity and prey preference in different prey availability contexts, we repeated Experiment 1 by keeping the mean prey density (24 prey in total), but changing the relative abundance of the two prey species, using five ratios between the prey species (3:21, 6:18, 12:12, 18:6, 21:3). For logistical reasons, each combination was replicated eight times in this experiment. At the same time, four replicates of predator-free controls at each prey ratio to quantify background prey mortality were performed. Thus, our experiment consisted of 15 treatment combinations (five ratios x three claw groups) with a total of 120 independent observations and 20 additional controls.

Statistical analysis

In both experiments, the total number of prey killed was analyzed using generalized linear mixed models (GLMMs) using the *glmer* function with a Poisson distribution and log link from the lme4 R package (Bates et al. 2014). The number of killed prey was modeled as the dependent variable, with prey species, crayfish group (i.e. claw group) and prey density (6, 12, and 24 for Experiments 1 and 3, 6, 12, 18, and 21 for Experiment 2) as fixed effects, their triple interaction, and replicate identity as random effect to account for non-independent measurements of the two prey types in each experimental arena. A Tukey post-hoc test was used to adjust for multiple comparisons and further explain significant effects using the emmeans R package (Lenth 2023).

For the prey preference at different prey proportions (Experiment 2), a selectivity index was calculated (Eq. 1; Manly 1974; Chesson 1983) to measure the preference of the predator *P. virginalis* for *G. fossarum* prey over *C. plumosus*. Adjustments were made to account for the non-replacement of prey.

$$\alpha = \frac{log10\left(\frac{n_i - r_i}{n_i}\right)}{log10\left(\frac{n_i - r_i}{n_i}\right) + log10\left(\frac{n_{n0} - r_n}{n_{n0}}\right)} \quad (1)$$

where α is the selectivity index for non-native *G. fossarum*, n_i is the number of the *G. fossarum* available at the start of the experiment, r_i is the number of the number of killed *G. fossarum*, n_{n0} the number of *C. plumosus* available at

the start of the experiment and r_n is the number of killed *C. plumosus*. The index ranges from 0 to 1, where 0 indicates complete avoidance (i.e. preference for *C. plumosus*), 0.5 neutral selectivity between prey species (i.e. no preference) and 1 indicates complete preference towards the *G. fossarum*. The resulting α values were transformed (Eq. 2) to account for extreme data points (0,1; Smithson and Verkuilen 2006).

$$\alpha_t = \frac{((\alpha * (n-1)) + 0.5)}{n} \quad (2)$$

where α_t is the transformed selectivity index and n is the sample size (i.e. 24). Finally, following this transformation, beta regression was performed using the *betareg* function of the lme4 R package (Cribari-Neto and Zeileis 2010) to compare indices over the different prey ratios and crayfish claw groups. Lastly, we performed an additional model with an interaction and compared with the previous model through the *lrtest* function of the lmtest R package (Zeileis and Hothorn 2002). All statistical analyses were conducted in R (v.4.2.3; R Core Team 2022).

Results

Experiment 1

No natural mortality of prey was observed in controls and therefore the results were not adjusted for background prey mortality. We found a significant interaction between the crayfish group and prey species (χ^2 = 10.34 ; p < 0.01; Table 1), with significantly fewer individuals of *G. fossarum* killed by clawless crayfish than by crayfish with one and both claws (p < 0.001; Fig. 1, Suppl. material 1: table S1). The triple interaction between the number of claws, prey species, and prey density was marginally significant (χ^2 = 5.84; p = 0.05; Table 1), at high prey density, crayfish with both claws appear to be able to kill more *C. plumosus*. In contrast, the number of claws did not affect chironomid predation (p > 0.05; Suppl. material 1: table S1). The number of killed prey positively depended on the total density of prey (χ^2 = 51.21; p < 0.0001; Table 1). Finally, the random effect of replicate identity was very weak (Variance = 0.05, SD = 0.23).

Table 1. Results of the generalized linear mixed effect model on the number of prey (*Chironomus plumosus* larvae and *Gammarus fossarum*) kept at three densities (6:6, 12:12 and 24:24) killed by clawless, one clawed, both clawed (crayfish groups) *Procambarus virginalis*. A random effect of the experimental arena was included. Df corresponds to the "Degrees of Freedom" and Pr(>Chisq) corresponds to the "Probability of the χ2". Significant values in bold.

	χ^2	Df	Pr(>Chisq)
(Intercept)	26.24	1	**< 0.0001**
Prey density	51.21	1	**< 0.0001**
Crayfish group	0.04	2	0.98
Prey species	22.68	1	**< 0.0001**
Prey density: Crayfish group	0.54	2	0.76
Prey density: Prey species	0.42	1	0.52
Crayfish group: Prey species	10.34	2	**< 0.01**
Prey density: Crayfish group: Prey species	5.84	2	0.05

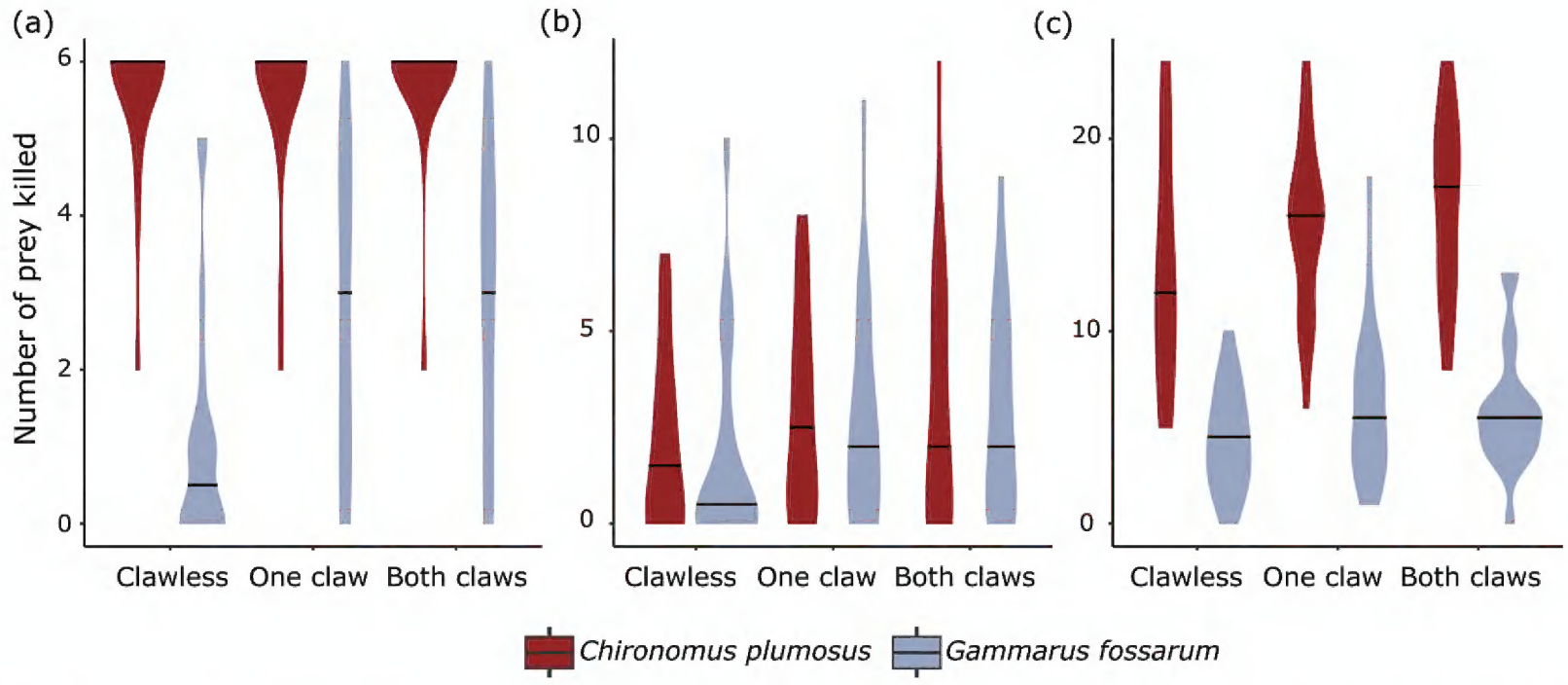

Figure 1. Number of prey (red: *Chironomus plumosus* larvae; grey: *Gammarus fossarum*) killed by *Procambarus virginalis* as a function of the prey density 6:6 (**a**), 12:12 (**b**) and 24:24 (**c**) and the number of predator's claws (clawless, one claw, both claws).

Experiment 2

As for Experiment 1, we did not adjust the results of Experiment 2 due to the low natural prey mortality (3.1% for *G. fossarum* and 1.0% for *C. plumosus*). The inclusion of prey density and number of claws improved the fit of the model to the data compared to the intercept-only model ($p = 0.03$). The number of killed prey was positively influenced by prey frequency density ($\chi^2 = 27.99$; $p < 0.0001$, Table 2) and by the prey species, with a preference towards chironomid larvae ($\chi^2 = 5.99$; $p < 0.05$, Table 2). Finally, the random effect of replicate identity was weak (Variance = 0.13, SD = 0.36).

The transformed selectivity index toward *G. fossarum* was lower than the null preference of 0.5 for the three crayfish claw groups, but at equal density (i.e. 12 *G. fossarum* and 12 *C. plumosus*), there was no clear preference for the crayfish group with both claws (Fig. 2, Table 3).

Moreover, we found that predation was frequency-dependent, particularly for proportions of 0.750 (18 prey of *G. fossarum* and 6 prey of *C. plumosus*) and 0.875 (21 prey of *G. fossarum* and 3 prey of *C. plumosus*) of *G. fossarum* where preferences disproportionately decreased for the gammarids compared to 0.5 ($p < 0.01$; Suppl. material 1: tables S2, S3). There was no shift in prey preference with the number of claws ($p = 0.95$ and 0.79; Suppl. material 1: table S2).

Table 2. Results of the generalized linear mixed effect model on the number of prey (*Chironomus plumosus* larvae and *Gammarus fossarum*) kept at 5 different ratios (3:21, 6:18, 12:12, 18:6, 21:3) and killed by clawless, one clawed or both clawed (crayfish groups) *Procambarus virginalis*. A random effect of crayfish identity was set. Df corresponds to the "Degrees of Freedom" and Pr(>Chisq) corresponds to the "Probability of the χ^2". Significant values in bold.

	χ^2	Df	Pr(>Chisq)
(Intercept)	6.16	1	**< 0.05**
Prey density	27.99	1	**< 0.0001**
Crayfish group	2.17	2	0.34
Prey species	5.99	1	**< 0.05**
Prey density: Crayfish group	1.53	2	0.47
Prey density: Prey species	1.74	1	0.19
Crayfish group: Prey species	0.58	2	0.75
Prey density: Crayfish group: Prey species	2.54	2	0.28

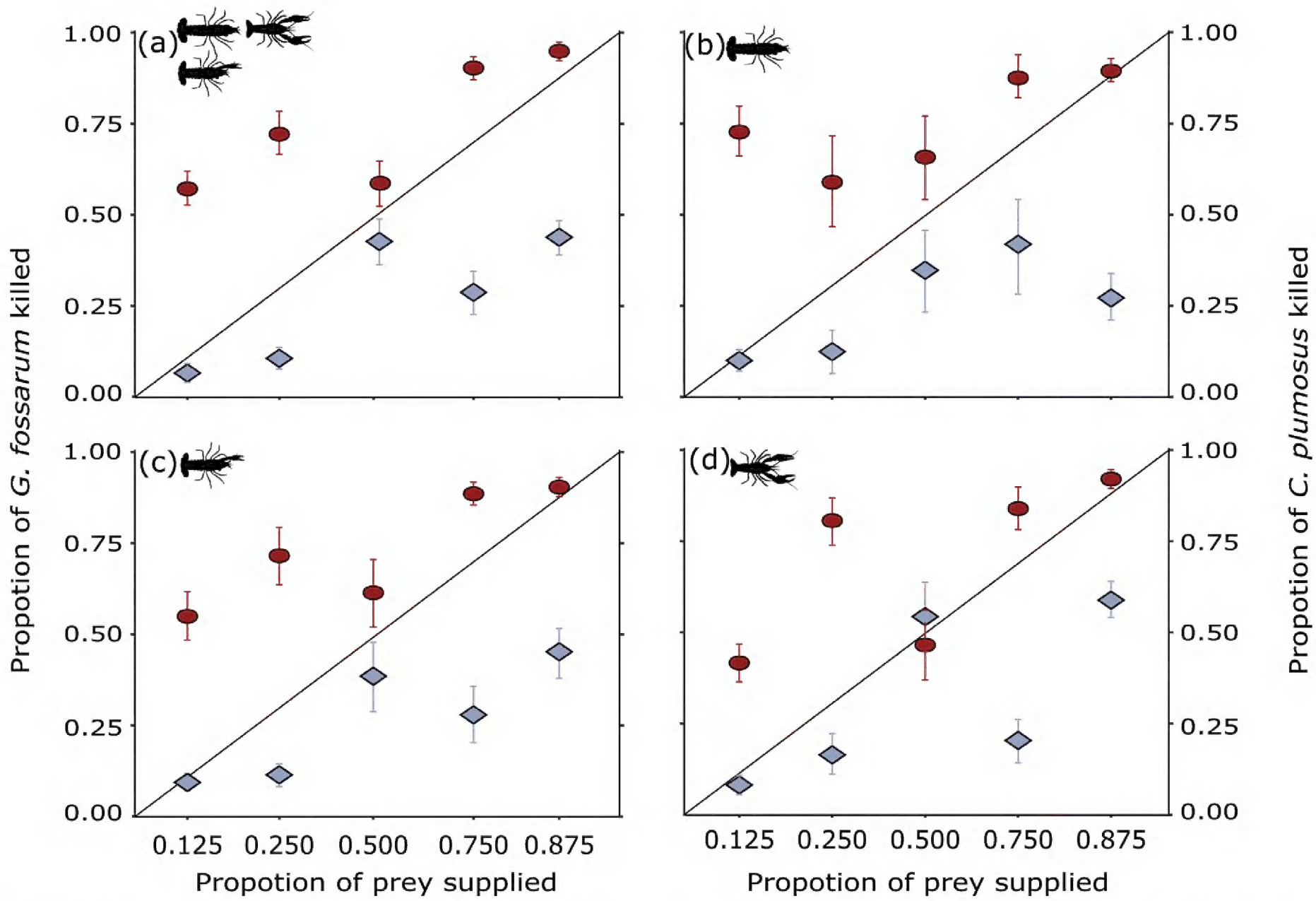

Figure 2. Proportion of prey killed as a function of the ratio of the provided prey (number of *Gammarus fossarum* over the number of *Chironomus plumosus* larvae) in *Procambarus virginalis*: total (**a**), clawless (**b**), with one claw (**c**) and with both claws (**d**). The grey rhombi indicate the average proportion (± S.E.) of *G. fossarum* killed and the red circles indicate the average proportion (± S.E.) of *C. plumosus* killed. The solid line (bisectrix) indicates the expected values if there was no preference between the two prey types.

Table 3. Selectivity indices (α_t ± S.E.) for *Procambarus virginalis* towards *Gammarus fossarum* or *Chironomus plumosus* larvae at varying prey ratios. Selectivity indices above 0.5 indicate a positive preference for *G. fossarum*, while indices below 0.5 indicate a preference for a given prey, whereas below 0.5 indicates avoidance of that prey".

Proportion of prey supplied	Prey type	All the crayfish groups	Clawless	One claw	Both claws
0.125 (Prey ratio 21:3)	***G. fossarum***	0.24 ± 0.05	0.23 ± 0.10	0.25 ± 0.09	0.22 ± 0.08
0.250 (Prey ratio 18:6)	***G. fossarum***	0.21 ± 0.05	0.17 ± 0.09	0.19 ± 0.06	0.27 ± 0.11
0.5 (Prey ratio 12:12)	***G. fossarum***	0.41 ± 0.06	0.34 ± 0.12	0.38 ± 0.10	0.49 ± 0.11
0.750 (Prey ratio 6:18)	***G. fossarum***	0.14 ± 0.06	0.28 ± 0.15	0.07 ± 0.05	0.05 ± 0.02
0.875 (Prey ratio 3:21)	***G. fossarum***	0.06 ± 0.01	0.03 ± 0.01	0.09 ± 0.02	0.04 ± 0.02
0.125 (Prey ratio 3:21)	***C. plumosus***	0.94 ± 0.01	0.97 ± 0.01	0.91 ± 0.02	0.96 ± 0.02
0.250 (Prey ratio 6:18)	***C. plumosus***	0.86 ± 0.01	0.72 ± 0.01	0.93 ± 0.05	0.95 ± 0.02
0.5 (Prey ratio 12:12)	***C. plumosus***	0.59 ± 0.06	0.66 ± 0.12	0.32 ± 0.10	0.51 ± 0.11
0.750 (Prey ratio 18:6)	***C. plumosus***	0.79 ± 0.05	0.83 ± 0.09	0.81 ± 0.06	0.73 ± 0.11
0.875 (Prey ratio 21:3)	***C. plumosus***	0.76 ± 0.05	0.77 ± 0.10	0.75 ± 0.09	0.78 ± 0.08

Discussion

Crayfish are known for their omnivorous and opportunistic diet (Nyström et al. 1996; Edmonds et al. 2011; Balzani et al. 2024). Accordingly, non-native crayfish populations can cause substantial damage to invaded ecosystems even at low abundances (Grimm et al. 2020; Veselý et al. 2021). A number of controlled studies have been conducted to evaluate the prey preference (Correia et al. 2005; Haddaway et al.

2012; Linzmaier et al. 2020, or predation rates, in crayfish using diverse food items (South et al. 2019; Veselý et al. 2019). However, they never considered different ratios of the provided prey items. We found that the number of *G. fossarum* killed was significantly lower than the number of *C. plumosus* killed when both prey were together and this difference depended on the relative availability of each prey. In past studies with *P. virginalis* and these prey species, similar results were observed regarding prey frequency and feeding rates. In Toutain et al. (2024), proportional predation rates were recorded to decrease with increasing *G. fossarum* densities, thus reaching an asymptote at high densities owing to an overabundance of resources. That study reported crayfish maximum feeding rates ranging from 1.05 to 2.47 prey per hour, corresponding to approximately 25 to 60 mg of *G. fossarum* per hour. A similar pattern in maximum feeding rates was observed in Soto et al. (2023a), where *C. plumosus* larvae were used as a single prey resource, with rates ranging from 7.87 to 9.09 prey per hour, corresponding to approximately 284 to 357 mg of *C. plumosus* per hour. However, it is important to note that those previous studies used single-prey systems with higher prey densities compared to the current study (prey densities in Soto et al. 2023a; Toutain et al. 2024: 1, 3, 6, 12, 18, 24, and 36 individuals).

The number of crayfish claws did not strongly influence the overall number of prey killed; however, the results suggest a shift in prey preference at certain prey ratios, with fewer *G. fossarum* being targeted when crayfish had no claws. Thus, these findings support our first hypothesis, indicating that the loss of claws would reduce the number of *G. fossarum* killed. This was also observed by Toutain et al. (2024), who reported that the number of claws in *P. virginalis* influenced the predation rate of *G. fossarum*. The study also showed that certain predatory behaviors varied according to claw numbers, with individuals possessing both claws exhibiting higher attack rates and lower handling times compared to clawless individuals. Moreover, these results corroborate our first hypothesis that the loss of claws would not significantly affect predation on softer species such as *C. plumosus*. Similarly, following the findings of Soto et al. (2023a), the number of claws did not significantly influence chironomid prey consumption. Therefore, although *P. virginalis* generally prefers *C. plumosus*, the number of claws has occasionally influenced prey preference.

Prey preference

According to optimal foraging theory (MacArthur and Pianka 1966), which describes how animals optimize their food choices, prey preferences are influenced by a multitude of factors determining their profitability. These factors include prey abundance and nutritional value, catchability, predator learning, predator ontogenic stage and physiological state, as well as, prey behavior (McNair 1979, Theurich et al. 2024). For instance, crayfish diet can change according to their ontogeny (Loya-Javellana et al. 1993). Juveniles and adults of the noble crayfish *Astacus astacus* (Linnaeus, 1758) have small and very distinct trophic niches, with young adults occupying an intermediate position between both and presenting the widest niche. Juveniles have their diet based on zoobenthos, which is less consumed by adults, while adults have an important component of their diet based on cannibalism of juveniles (Veselý et al. 2020). The analysis of prey choice provides new insights into the understanding of *P. virginalis*' predator-multi-prey dynamics in invaded aquatic ecosystems. We found that in all situations, *P. virginalis* showed a strong preference for *C. plumosus* over *G. fossarum*. This food preference

can be attributed to several factors, such as the softness of the prey, its limited mobility, and ease of capture and handling. Compared to gammarids, which have a relatively tough carapace, chironomid larvae are, as many aquatic macroinvertebrates (Pinder 1986; Hein and Mahadeva 1988; Lencioni et al. 2018), soft prey that require less time and energy to be handled and consumed. Furthermore, mobile prey like *G. fossarum* are more difficult for *P. virginalis* to capture despite the absence of shelters and deep substrate (Jermacz et al. 2017, 2022). Nevertheless, it was already tested that the Northern clearwater crayfish *Faxonius propinquus* (Girard, 1852) with non-functional claws were able to consume mobile prey such as stoneflies and freshwater snails (Keller and Hazlett 1996; Gherardi et al. 2000). This predatory capability in the absence of the functional claws may be correlated with the use of the second and third pairs of pereiopods, which have smaller, yet sufficiently sized, claws. However, quantification of their involvement in foraging capacities requires further testing. Additionally, it should be mentioned that the larvae of *C. plumosus* were larger than *G. fossarum* and that *P. virginalis* were accustomed to regularly consuming thawed chironomid larvae in their original culture, which may have introduced a bias, either by promoting consumption due to familiarity or with *P. virginalis* possibly preferring larger prey. On the other hand, amphipods are arguably a richer source of calcium and play a significant roles in crayfish nutrition (Xu et al. 2021). We also note that, in more complex ecological settings, the absence of claws could shift foraging strategies of crayfish towards more risk-averse consumptive tactics or into different habitat types. This could result in a shift in preference towards lower-risk prey, owing to their compromised defenses, or a preference to forage in less complex habitats that are better suited to claw absence. These contexts could be further addressed using community module approaches, which simultaneously modulate trophic structure and levels of habitat complexity (Paterson et al. 2015).

Food preferences of non-native species can have major repercussions on ecosystem dynamics, as these non-native species have often not co-evolved with their potential prey. When predators show a preference for one or more prey species without adopting target-switching behavior, this can have significant consequences for the population of the preferred prey because low-density populations cannot be recovered (Schoener et al. 2001; Cuthbert et al. 2018). The strong feeding preference of *P. virginalis* for *C. plumosus* could thus significantly reduce their populations and those of similar species, leading to profound changes in food webs, energy flows and biogeochemical cycles (Fukuhara and Sakamoto 1987; Hansen et al. 1997). Nevertheless, the transferability of these results to the field requires consideration.

Claw importance across different prey availabilities

Crayfish claws play an important role in intraspecific interactions (Graham and Angilletta 2020; Roje et al. 2021) and enable them to predate on other species (Keller and Hazlett 1996; Claussen et al. 2008; Sanders and Mills 2022). The loss of claws generally depends on a crayfish population's density, as it directly affects the likelihood of encounters among individuals (Savolainen et al. 2003; Graham et al. 2021), but little information is available on the ecology of *P. virginalis* (Linzmaier et al. 2020; Lipták et al. 2019), and the incidence of claw injury has been rarely noted. Maciaszek et al. (2022) described a relatively small proportion of the population (around 8%) having claws missing or regenerating, although this value can vary

between populations and interacting species (Kouba et al. 2021; Veselý et al. 2021). *Procambarus virginalis* is generally considered a suitable model species that is unaggressive, tolerating relatively high culture densities (Hossain et al. 2018, 2019) with claws rarely damaged when kept in intraspecific stocks (Kouba et al. 2021). Our results show that *P. virginalis* was able to modify its preference for prey according to the number of claws, but generally consumed a greater amount of *C. plumosus* than of *G. fossarum*, regardless of the number of claws or the relative availability of prey. Although crayfish claws are important for capturing mobile, rather hard-shelled prey like *G. fossarum* (Toutain et al. 2024), their role appears less crucial when dealing with less mobile, soft prey such as *C. plumosus* (Soto et al. 2023a). However, this distinction does not fully account for the previously mentioned lack of claw effect, suggesting that other factors may also influence prey capture efficiency. Therefore, the presence of such soft-bodied prey might compensate for the effect of claw loss in crayfish, but the evidence here was ambivalent. The strong preference of *P. virginalis* for soft and immobile prey, such as *C. plumosus*, over harder, mobile prey, such as *G. fossarum*, can lead to an imbalance in food webs and endanger some native species (Sánchez et al. 2024). These effects will also depend on the extent of familiarity with invasive predators and prey in their novel range (Cox and Lima 2006; Sih et al. 2010). Nevertheless, the low importance of claws in the predation of soft prey suggests that even individuals that have suffered claw losses remain effective predators.

Conclusions

Food preference of *P. virginalis* was principally for soft and less mobile prey such as *C. plumosus*, as contrasted with more mobile and harder prey such as *G. fossarum*. As a result, this study showed that the impact of crayfish predation also depended on the frequency of autotomized animals and the availability of prey, although *P. virginalis* showed a strong preference for *C. plumosus*. While *P. virginalis* offers a useful baseline model for investigating the effects of claw loss under controlled conditions, its use also provides insights into the general role of claws in feeding and consumption behaviors across crayfish species with varying morphology and biometry. To evaluate the broader ecological relevance and functional consequences of claw loss, future studies should extend this approach to ecologically distinct, sexually reproducing, and morphologically diverse taxa. Non-native species with larger claws, such as the signal crayfish *Pacifastacus leniusculus* and the red swamp crayfish *P. clarkii*, are of particular interest due to their size and voracity, and it would be interesting to test their response to even larger prey, such as mollusks or small fishes.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization: PB, MT, AK. Data curation: IS, MT. Formal analysis: RNC, MT. Funding acquisition: AK, MT. Investigation: AK, MT. Methodology: FJO, AK. Resources: AK. Supervision: AK. Validation: AK. Visualization: MT. Writing - original draft: FJO, AK, PB, IS, RNC, MT, AFH. Writing - review and editing: FJO, IS, PB, AK, AFH, RNC.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary data

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Data type: docx

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